

Microorganisms Found on Spacecraft and Associated Surfaces: Preservation, Identification, Characterization

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Project Objective: To archive incoming isolates from planetary protection bioassays and to identify those isolates using MALDI-TOF mass spectrometry and 16S rRNA identification

Background on Planetary Protection

- Aims to prevent the forward contamination of microorganisms between Earth and extraterrestrial bodies
- NASA Standard Spore Assay is used as a proxy to measure the microbial bioburden present on spacecraft hardware and associated surfaces.
- Microbes isolated as a result of this assay, are archived for long term preservation, documented, and identified.

Methods for Identification of Organisms

- Isolates were run on the MALDI-TOF (matrix assisted laser desorption/ionization time-of-flight) mass spectrometer
- Organism was initially run using RTC (real time classification), which compared organism spectra against the JPL in-house database
- If organism did not match to the database, an MSP (mass spectral profile) was added to the database for the organism and the MSP was named according to 16S rRNA identification
- Quality control measures in place ensured that adequate spectra were used to create an MSP (at least 10 good spectra needed)

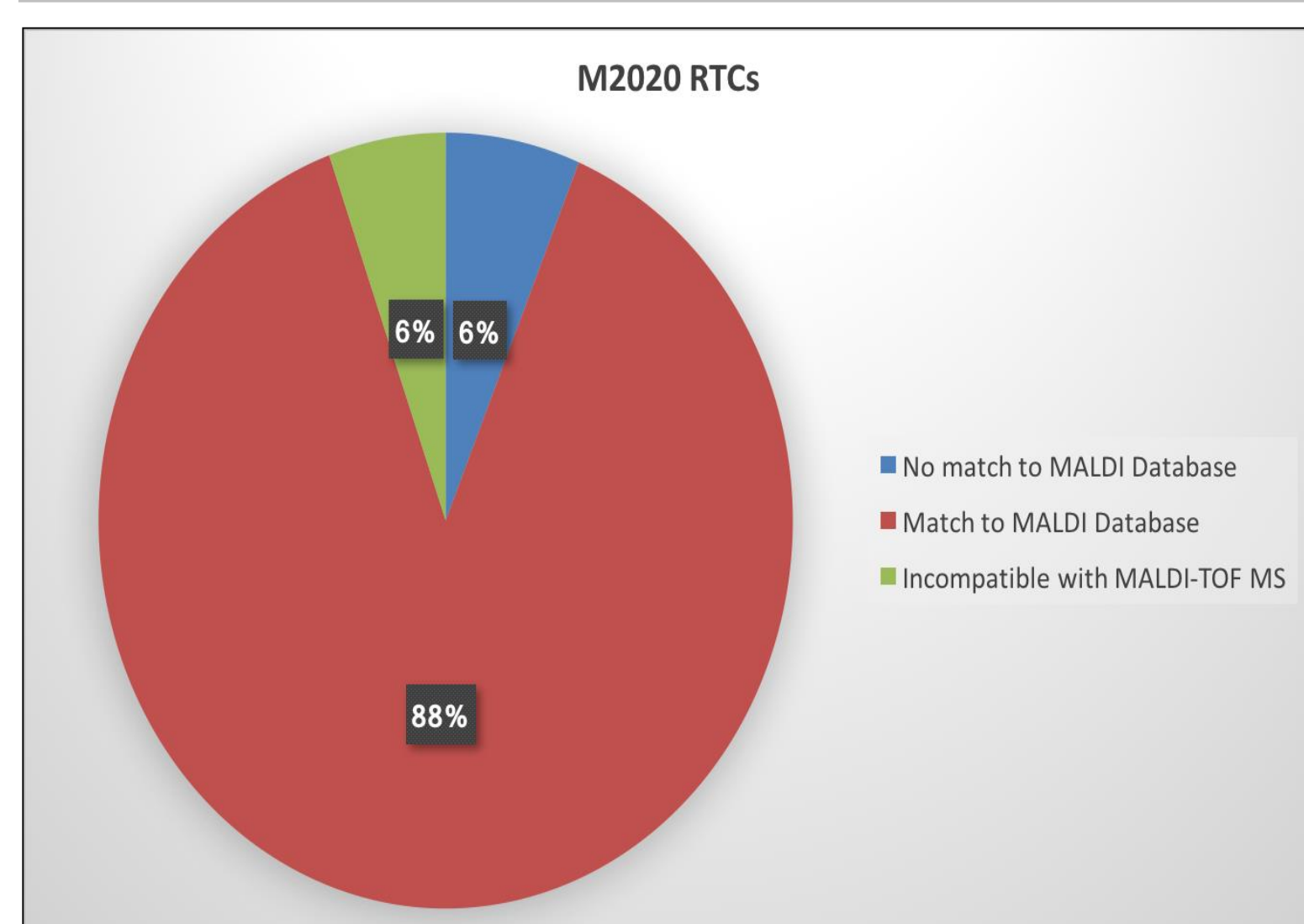


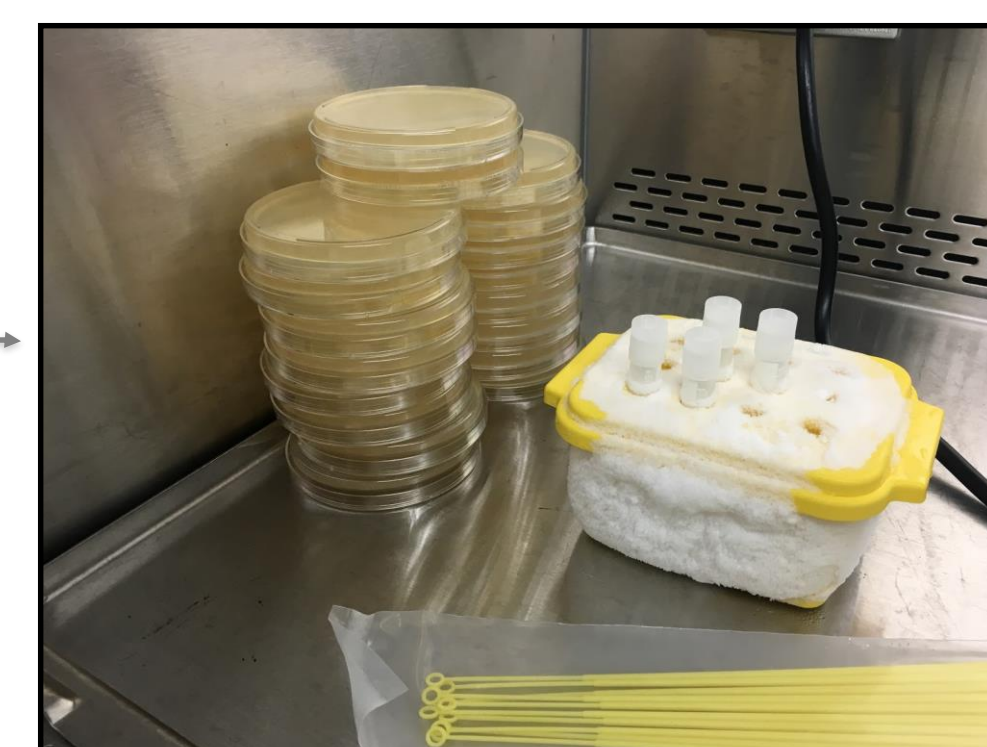
Figure 2: A chart showing the results of all M2020 isolates when compared against the database. 88% matched to a spectra already in the database, 6% could not be run because of poor morphology, and 6% required MSP creation.

Results

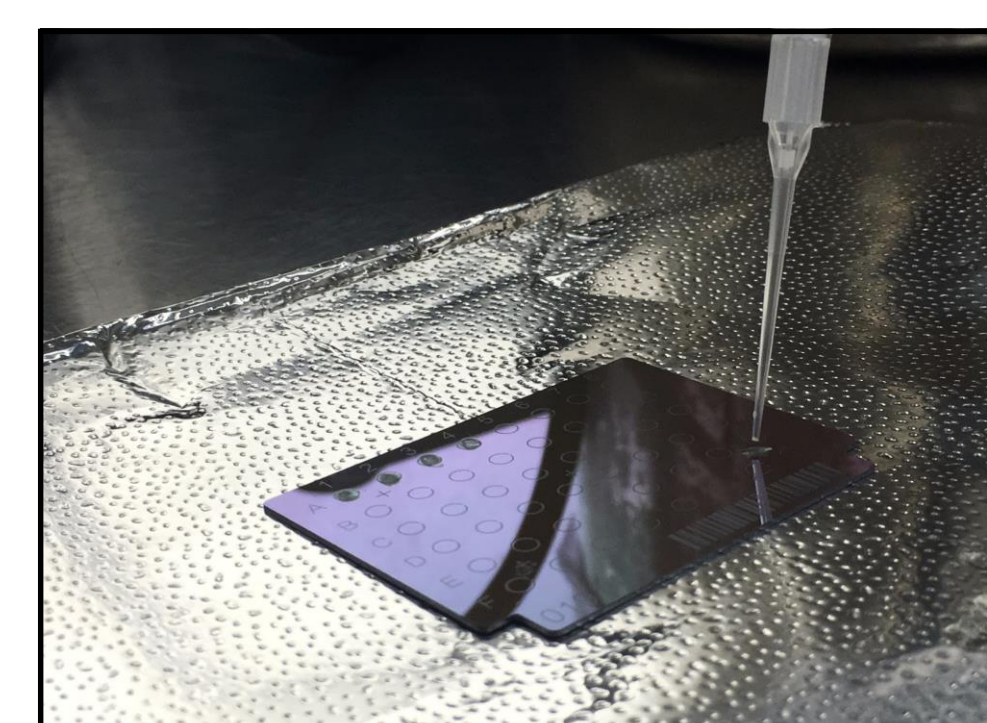
- 135 new M2020 isolates archived and identified this summer
- 40 MSP's created for those M2020 isolates that did not match to the database
- 55 MSP's renamed in the database: spectra re-evaluated and MSP named correctly according to 16S ID
- 24 MSP's from the database were remade because they were created with poor spectra in the past

Future Directions

- Continually maintain and update an in-house database that encompasses the microbial diversity on spacecraft and associated surfaces
- Continue archiving, identifying, and characterizing samples collected from spacecraft and associated surfaces



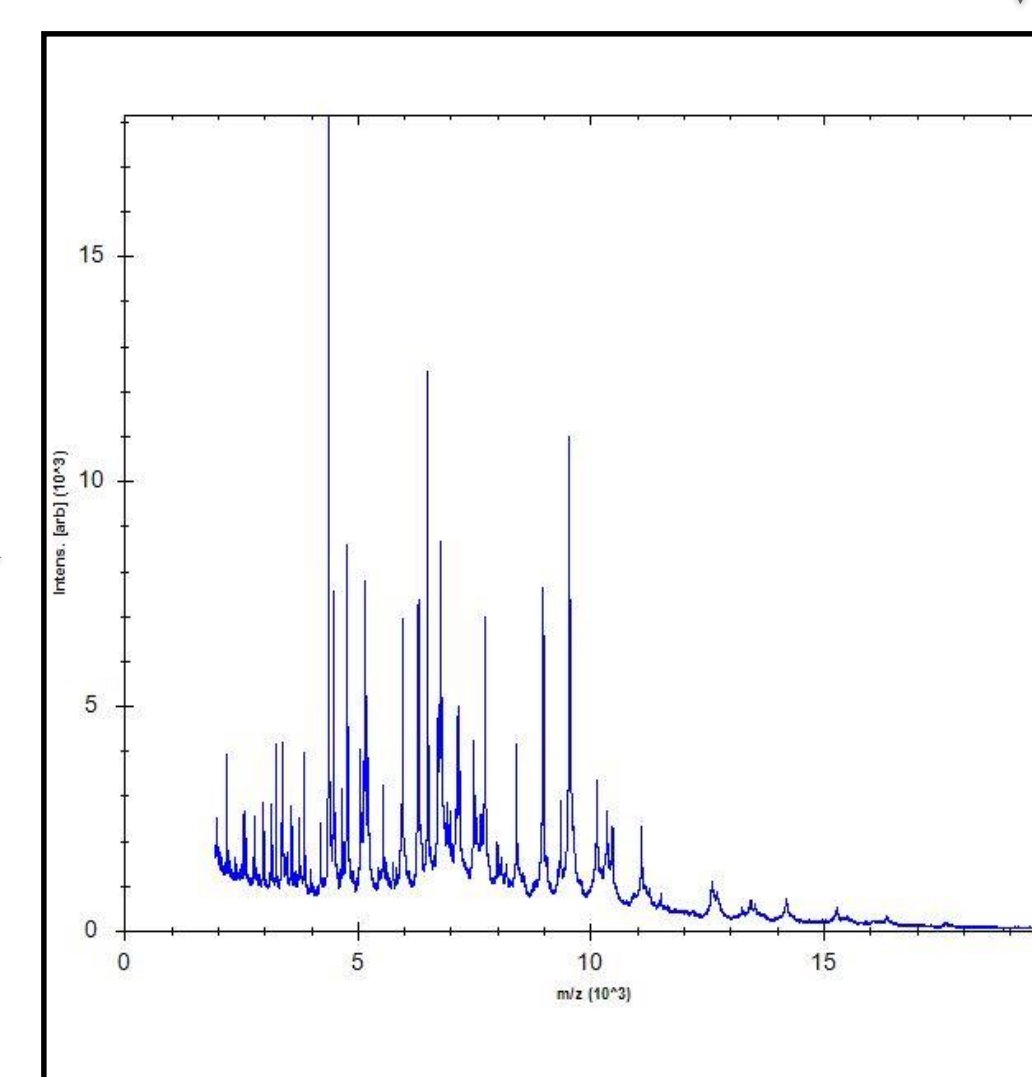
Subculture organism from bioassay plate or revive from stock



Streak organism on target, lyse with formic acid, overlay with matrix, and calibrate with BTS



Run RTC on MALDI-TOF mass spectrometer; RTC compares an isolate's protein spectrum against the in-house database



If organism does not match to database, create MSP, send isolate out for sequencing, and then add MSP to database using the name from 16S ID

Figure 1: A flowchart showing how an isolate is identified using the MALDI-TOF mass spectrometer.